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MOLECULAR CHARACTERISATION OF TETRACYCLINE RESISTANCE OF SOME AVIAN *SALMONELLA TYPHIMURIUM* AND *SALMONELLA ENTERITIDIS* STRAINS

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Genetic screening by hybridization with *tet M* and *tet O* DNA probes demonstrated, in *Salmonella* strains isolated in 1996, the following: 14/15 (93.3%) of *S.enteritidis* strains and 9/12 (75%) of *S. typhimurium* strains harbored the *tet M* determinant; 10/15 (66.6%) of *S. enteritidis* strains and 7/12 (58.3%) of *S. typhimurium* harbored also the *tet O* determinant.

The PCR screening, for which we used primers provided by Dr. Elisabeth Chaslus Dancla, INRA Tours, France, and which underline a common fragment for the A,B,C,D,E determinants, was negative.

The presence of the *tet M* and *tet O* determinants, which code the resistance mechanism by ribosomal protection against all types of tetracycline (first generation and the analogues of the second generation) could be attributed to a genetic acquisition from Gram-positive bacteria.

In conjugation tests, with *E. coli* BM21 (*gyr A*, Nalr), tetracycline resistance shown high transfer frequency, which suggests the possibility of high epidemiological risk. The origin of strains – three Romanian geographical regions – suggests that the M and O tetracycline resistance determinants were widespread among avian *S. typhimurium* and S. *enteritidis* strains.

***Key words**: Poultry, tetracycline, resistance, hybridisation, PCR, tet O, tet M*

INTRODUCTION

A recrudescence of *Salmonella* strains isolations has been recorded in recent years in the poultry flocks of the Romanian avian industry: their frequency that was, in the Avian Pathology Department, Pasteur Institute, Bucharest, 7% in 1993 (5) reached 21% in 1996. Also, the resistance to tetracyclines is constantly rising: its relative frequency, in the avian *Salmonella* isolates, of 45% in 1993 reached 64% in 1996. The necessity arose worlds wide, for a surveillance programme for the microbial antibioresistance in animals with a view to, among others, rise on rational bases certain feed additives, and the implications of possible transfer of this resistance to humans (4,8). The standard control methods cannot be replaced,

but as the data are on many occasions ambiguous, the methodology of molecular biology is resorted to. The molecular characterisation of the bacterial strains is indispensable to a high quality epidemiologic study of the pathogenic strains on raising farms, and to their more rigorous control. In other words, the epidemiological surveillance is assisted by molecular typing techniques (6,11).

As the resistance to tetracycline may be considered a dominant phenotypic character of the avian *Salmonella* strains in the Pasteur Institute's collection we set ourselves the task to molecularly and genetically characterize it.

Although there exist almost 100 tetracycline derivatives, only 7 are extensively used in the veterinary practice. The representatives described between 1948 - 1957 constitute the first generation, those described between 1965 - 1972 being the second generation. Tetracyclines were the first major group of antimicrobial agents in connection with which the term "broad spectrum" was used as they are active against various Gram-positive and – negative bacteria, and even against some parasitic protozoa. They have therefore been used in the therapy of human and animal infections, even in those of plants and insects. But the most important limit of their usage is the microbial resistance developed against these agents.

The bacterial resistance to the tetracyclines of clinical use is mainly caused by the resistance acquired through the occurrence of resistant strains in a bacterial population that was initially sensitive based on the selective pressure of the very administration of tetracyclines for chemotherapeutic purposes. The genes determining the resistance to tetracyclines commonly harbour mobile genetic elements like plasmids and/or transposons; the later may, in their turn, be either complex transposons, constituents of the Tn3 family or conjugative ones (13). The acquisition of the determinants of the resistance on plasmids and transposons is important particularly in the evolution of the antibioresistant bacteria as they provide the receptor cell predeveloped genes useful in the development of the high level expressions of resistance (3). The biochemical mechanisms of the resistance to tetracyclines are the: a) energy - dependent efflux of the antibiotic mediated by the resistance proteins localised into the bacterial cytoplasmatic membrane; b) ribosomal protection by which tetracyclines can no more bind to the bacterial ribosome (site A of subunit 30S), and c) chemical alteration of the tetracycline molecule by means of an oxygen - requiring reaction that inactivates the drug as an inhibitor of the proteinic synthesis. This last mechanism may exist under an unexpressed form if the infectious foci in bird's body are poorly aerated (3,10).

The determinants of the resistance to tetracyclines have been grouped in definite classes based in the lack of cross-hybridisation under stringent conditions. However, some classes are related as they encode products with similar functions, such as the determinants mediating the tetracyclines efflux out of cell. Some of the resistance determinants, such as *tet* B and *tet* M, confer resistance both to the first and the second generation analogues of tetracyclines, while others, i.e. *tet* A, C,D,K,L, offer poor protection to the second generation analogues, particularly to minocycline. Incidentally, the *tet* determinants may induce poor resistance; they may even not protect against the tetracyclines analogues whose main target is the cytoplasmatic membrane. Some determinants, of the *tet* A - E type, contain a structural gene and a repressor one that mediate the resistance to tetracyclines. Although the determinants of the resistance to tetracyclines are widely spread in pathogenic and non-pathogenic bacteria, certain classes seemed, up to a certain moment, to be more frequent in certain microbial groups; the explanation was the natural restrictive

acquisition of these determinants in bacterial populations at least two types of resistance mechanisms may be expressed in the same cell (3,9,10).

The evaluation of this resistance - associated epidemiologic risk involves, besides evidencing the transfer frequency level, the determination as well of the genetic determinants classes prevalence in the bacterial strains under study.

Materials and methods

Avian bacterial strains of *Salmonella cholerae suis*, serovars *typhimurium* and *enteritidis*, under study originate from the isolations conducted in 1996 at the Central Veterinary Diagnosis Laboratory, Bucharest, and at the Avian Pathology and Molecular Biology Departments, the Pasteur Institute, Bucharest. The classification into the phagic type was carried out at the National Reference Centre for Salmonella, the Cantacuzino Institute, Bucharest. The investigations were performed by means of 18 hours old cultures in BHI (*Sanofi Diagnostic Pasteur*).

The antibiophenotype was determined by Kirby - Bauer diffusimetry (1), for: ampicillin (Amp), amoxicillin (Amo), chloramphenicol (Cm), Kanamycin (Km), streptomycin (Sm), tetracycline (Tc), doxicycline (Dc), minocycline (My), gentamycin (Gm), nalidixic acid (Nal), and sulphonamide (SSS) (biodiscs *Sanofi Diagnostic Pasteur*, Mueller Hinton culture medium *Sanofi Diagnostic Pasteur*).

The resistance to tetracyclines was self-transferred by obtaining transconjugants with E. coli K_{12} BM_{21} (*gyr* A, Nal^r) as receptor strain (11). The conjugations were in liquid medium (11), or on nitrocellulose membranes with hydrophobic contour (HAEP, Millipore), by Buu - Hoï & Horodniceanu technique of 1980 (13). Based on this technique, the transfer frequency was calculated by means of the ratio between the CFU/ ml of the transconjugants and the CFU / ml of the donor cells. The high transfer frequency ranges between 10^{-1} and 10^{-4}, and the low one from 10^{-5} to 10^{-9}.

Molecular characterisation

Plasmidic DNA extraction and analysis

The plasmidic material was isolated based on the technique described by Takahashi and Nagano, 1984 (14), and analysed as previoulsy reported (6,11).

Molecular screening for tet determinants

Tet probes preparation

The plasmids of interest in the reference strains containing plasmids recombined with the *tet* sequences were purified by ultracentrifugation in caesium chloride (Protocol, Pasteur Institute, Paris).

Following the agarose gel electrophoresis with low fusion point, the double enzymatic hydrolysis was performed after which the bands from the consecutive electrophoresis were digoxigenine labelled (*Dig-High Prime, Boehringer Mannheim*) as per the manufacturer's Protocol. The *tet* M probe was prepared and labelled at INRA, Tours, France.

Demonstration of tet determinants by membrane hybridisation

The genetic material was either extracted in keeping with Wilson's method, 1987 (16) and used in the hybridisation dot-blot technique, or evidenced follwing the colonies transfer directly on the membrane (12, 15).

Both membrane types were prehybridised with SSC 6x (SSC 1x : 0.15 M NaCl, 0.015M Na citrate), 0.5% SDS, 0.05% Blotto (5% skim Regilait milk in water), at 65oC overnight. Hybridisation was then applied in SSC solution 6x, 0.1% SDS, 0.05% Blotto with the digoxigenine labelled probe, at 65oC overnight. The membrane was then washed 3x at 65oC under stingency conditions with SSC 2x, SSC 2x-0.1% SDS, and SSC 0.1x (12). The developing was by Dig High Nucleic Acid Detection Kit (Boehringer Mannheim), as indicated by the manufacturer, with the exception of stop buffers that were replaced by deionised water.

The PCR screening for "tet" determinants

The primers used, "tet1" and "tet 3" originate from INRA Tours, France. They amplify a fragment of 600 pb considered as common for determinants A, B, C, D, and E and their sequence is: tet1 - 5'-ATG CCA GTA CTC CCT-3'; tet3 - 5'-TTG CCC GAT AAG CTG-3'.

They were selected and synthesised by the team of SPAP, INRA Tours, France. The DNA polymerase (Taq polymerase) was *Appligene,* and the oligonucleotides (dNTP)- *Gibco* (2mM each). The protocol used was: 1 cycle: 94°C-2', 55°C-1', 72°C-1.5'; 30 cycles: 94°C-1', 55°C-1', 72°C-1.5'; 1cycle: 72°C-10' and then indefinitely at 4°C in a reaction volume of 25ml using thermal cycler *GeneAmp PCR System 9600-Perkin Elmer.*

The amplification product was controlled by 1.5% agarose gel electrophoresis, 100V, 1h, ladder Raoul (Appligene) or Biozyme (*Gibco).* The reference strains containing the recombinant *tet* plasmids A, B,C,D,E and O of which the plasmid DNA had been extracted in agreement with the Takahashi and Nagano method were used as positive amplification controls.

Results and discussions

The strains - except for *S. typhiumurium* strain 407 that was isolated from a goldfinch and *S. enteritidis* strain 359 isolated from a calf (annex on a poultry farm)- originated from poultry flocks raised in the large scale pyramidal system in three geographical areas in five districts of Romania (figure 1).

Phenotypically, the strains fell into several resistance profiles (tables 1, 2, 1.2, 2.1, figures 2, 3).

Both serovars are characterised by the predominant pluriresistance to antibiotics(9/12-75% in *S. typhimurium* and 8/15-53.3% in *S. enteritidis*). In tetracyclines resistances group, only 5 *S.typhiumurium* strains were also resistant to doxycycline (one of them proving to be intermediary to minocycline as well).

On the whole no phenotypic expression of the resistance to minocycline was recorded.

Genetically, two strains of those tested for the self transferability of the resistance to tetracycline, i.e. *S. enteritidis* 358 and *S. typhimurium* 394 did not generate trans-conjugants by the liquid medium conjugation technique. The transfer frequences, determined by the membrane conjugation technique, recorded high values (10^{-1}-10^{-4}). With other

bacterial genera, the two techniques evidence the narrow or broad spectrum of hosts for the conjugative plasmids (13). The high transfer values and the results of the conjugation tests suggest, with the *Salmonella* strains analysed, that some of their plasmids might have a broader host spectrum.

Also, the plasmid co-transfer phenomenon was noticed with some strains, as demonstrated by plasmid profile of the transconjugants (small and large size plasmids). The plasmid profile, however, asks for additional molecular investigations.

The molecular screening based on hybridisations with probes *tet* M and *tet* O evidenced the following: 14 / 15 *S. enteritidis* strains and 9/12 *S. typhimurium* strains exhibited the presence of the *tet* M determinant, while 10 / 15 *S. enteritidis* strains and 7 / 12 *S. typhimurium* strains were positive for the *tet* O determinant as well (tables 1, 2, 1.2, 2.2, figures 4, 5). Strains 399 and 400 of *S. typhimurium* were negative for both types O and M determinants of the resistance to tetracycline. As mentioned in introduction, the simultaneous existence in the same strain of several determinants is not a new phenomenon; in the case of the *Salmonella* strains the hypothesis might be set forth of an acquisition of these determinants, initially residing in popuations of Gram-positive germs (*Enterococcus).*

Also, the prevalence of the two types of determinants - that induce resistance, through ribosomal protection, to most tetracyclines - indicates the risk of using an inefficient antibiotherapy in infections associated with these strains of *Salmonella.*

This risk should be mentioned the more so as certain fragments ot the tet determinants did not prove, in phenotypic tests, functional - this being suggested by the lack, in antibiograms, as compared to the molecular evidence, of the resistances to minocycline; the detectable expressed ones - they may also entail the mute ones transfer themselves at high frequences.

The origin of the strains in the three geographical areas (5 districts) suggests that the two determinants (M and O) of the resistance to tetracycline are widely spread in the avian strains of *S. typhimurium* and *S. enteritidis*.

The PCR molecular screening with *tet* 1 and *tet* 3 primers of the fragment estimated to be common to determinants A,B,C,D and E, proved negative for the test strains, including the two strains that had been negative for determinants M and O as well.

Table 1. *Salmonella enteritidis: origin, antibiotype, plasmidic profile, "tet" determinants*

No	Symbol	Strain / Serovar	Species / area	Antibiotype	Plasmidic Profiles Kb	Hybridization tet O	Hybridization tet M	"tet ABCDE" PCR
1	347	S. enteritidis	Poultry / BC	Tc^r	51.3, 4.3	+	+	-
2	350	S. enteritidis	Turkey / BC	Tc^r	51.3,3.9,3.5	+	+	-
3	357	S. enteritidis	Poultry / BC	Tc^r	75.8	-	+	-
4	358	S. enteritidis	Poultry / BC	Tc^r	66.1	-	+	-
5	359	S. enteritidis	Calf / BC	Tc^r	51.3,2.4	-	+	-
6.	360	S. enteritidis	Poultry / BC	Tc^r	51,3,3.4	-	+	-
7	363	S. enteritidis	Poultry / BC	Tc^r, SSS^r	51.3, 3.6, 3.4	+	+	-
8	366	S. enteritidis	Poultry / BC	Tc^r,Km^r,Sm^r	79.4, 3.8	+	+	-
9	368	S. enteritidis	Poultry / BC	Tc^r,Km^r	51.3	-	+	-
10	372	S. enteritidis	Poultry / IF	Tc^r	51.3, 3.6, 2.4	+	+	-
11	373	S. enteritidis	Poultry / IF	Tc^r	75.8, 3.8, 3.3	+	+	-
12	385	S. enteritidis	Poultry /MM	Tc^r,Am^r,Cm^r, Amo^r,Dc^r (My)	51.3,4.3,3.5,3.3,2.4	+	+	-
13	396	S. enteritidis	Poultry / BR	Tc^r, SSS^r	51,3,3.6	+	+	-
14	402	S. enteritidis	Poultry / BR	Tc^r, SSS^r	75.8,3.8,3.3	+	+	/
15	404	S. enteritidis	Poultry / BR	Tc^r,Am^r,Cm^r	66,4.8,3.6,3.4,2.9	+	+	/

Table 2. *Salmonella typhimurium: origin, antibiotype, plasmidic profile, "tet" determinants*

No	Symbol	Strain / Serovar	Species / area	Antibiotype	Plasmidic Profiles Kb	Hybridization tet O	Hybridization tet M	"tet ABCDE PCR
16	338	S. typhimurium	Poultry / CS	Tc^r	63,3.6,3.5	+	-	/
17	383	S. typhimurium 36	Poultry /MM	Tc^r,SSS^r,Sm^r	131.8,91.2	-	+	-
18	384	S. typhimurium 36	Poultry /MM	Tc^r,SSS^r,Sm^r	100,95.5	-	+	-
19	394	S. typhimurium 120	Poultry / BR	Tc^r,Km^r	91.2	-	+	-
20	399	S. typhimurium 1	Poultry / BR	Tc^r, SSS^r	91.2,5.3,5	-	-	-
21	400	S. typhimurium 1	Poultry / BR	Tc^r	100,3.6	-	-	-
22	407	S. typhimurium nt	Goldfinch / BR	Tc^r,Gm^r,Cm^r, SSS^r,Dc^r	131.8,91.2,3,2.5	+	+	-
23	408	S. typhimurium nt	Poultry / BR	Tc^r,Gm^r,Cm^r, SSS^r,Dc^r	100,95.5,2.9,2.6	+	+	-
24	409	S. typhimurium nt	Poultry / BR	Tc^r,Gm^r,Cm^r, SSS^r,Dc^r	100,95.5,2.9,2.6	+	+	-
25	414	S. typhimurium 1	Poultry / BR	Tc^r	131.8,91.2,4.6,4.4	+	+	-
26	415	S. typhimurium 1	Poultry / BR	Tc^r,Gm^r,SSS^r, Dc^r	131.8,91.2,2.7,2.5	+	+	-
27	416	S. typhimurium 1	Poultry / BR	Tc^r,Gm^r,SSS^r, Dc^r	131.8,91.2,3,2.5	+	+	-

table 3. *Salmonella enteritidis: Transconjugants (x E.coli BM_{21}), plasmidic profile, tetracycline resistance transfer frequency*

No	Symbol	Donor Serovar	Antibiotype	Plasmidic Profiles Kb	Transconjugant(x BM_{21}) liquid medium Abtype	Transconjugant(x BM_{21}) liquid medium PP(Kb)	"tet" RTF membran
1	347	S. enteritidis	Tc^r	51.3, 4.3	ND	ND	ND
2	350	S. enteritidis	Tc^r	51.3,3.9,3.5	Tc^r	51.3	$7x10^{-4}$
3	357	S. enteritidis	Tc^r	75.8	Tc^r	75	$3x10^{-3}$
4	358	S. enteritidis	Tc^r	66.1	-	-	ND
5	359	S. enteritidis	Tc^r	51.3,2.4	Tc^r	51.3	$5x10^{-6}$
6.	360	S. enteritidis	Tc^r	51.3,3.4	Tc^r	51.3	$3x10^{-2}$
7	363	S. enteritidis	Tc^r, SSS^r	51.3, 3.6, 3.4	Tc^r	51.3, 3.5	$2x10^{-1}$
8	366	S. enteritidis	Tc^r,Km^r,Sm^r	79.4, 3.8	Tc^r,Km^r	79.4	$3x10^{-4}$
9	368	S. enteritidis	Tc^r,Km^r	51.3	Tc^r,Km^r	51.3	$5x10^{-2}$
10	372	S. enteritidis	Tc^r	51.3, 3.6, 2.4	Tc^r	51.3, 3.5	$3x10^{-5}$
11	373	S. enteritidis	Tc^r	75.8, 3.8, 3.3	Tc^r	75	$1x10^{-2}$
12	385	S. enteritidis	Tc^r,Am^r,Cm^r, Amo^r,Dc^r (My)	51.3,4.3,3.5,3.3,2.4	ND	ND	ND
13	396	S. enteritidis	Tc^r, SSS^r	51,3,3.6	Tc^r	51.3	$5x10^{-2}$
14	402	S. enteritidis	Tc^r, SSS^r	75.8,3.8,3.3	Tc^r	75, 3.5	$3x10^{-3}$
15	404	S. enteritidis	Tc^r,Am^r,Cm^r	66,4.8,3.6,3.4,2.9	ND	ND	$1x10^{-2}$

Tc – tetracycline Km – kanamycin Sm – streptomycin Am – ampicillin Amo – amoxicillin Cm – chloramphenicol Dc – doxicyclin SSS – sulfamide

Table 4. *Salmonella typhimurium: Transconjugants (x E. coli BM_{21}) plasmidic profile, tetracycline resistance transfer frequency*

No	Symbol	Donor Serovar	Antibiotype	Plasmidic Profiles Kb	Transconjugant (x BM_{21}) liquid medium Abtype	Transconjugant (x BM_{21}) liquid medium PP(kb)	"tet" RTF membran
16	338	S. typhimurium	Tc^r	63,3.6,3.5	Tc^r	63,3.6,3.5	$2x10^{-2}$
17	383	S. typhimurium 36	Tc^r,SSS^r,Sm^r	131.8,91.2	Tc^r, Sm^r	131.8, 91	$3x10^{-2}$
18	384	S. typhimurium 36	Tc^r,SSS^r,Sm^r	100,95.5	Tc^r, Sm^r	100, 95.5	$1x10^{-1}$
19	394	S. typhimurium 120	Tc^r,Km^r	91.2	-	-	ND
20	399	S. typhimurium 1	Tc^r, SSS^r	91.2,5.3,5	Tc^r	91.2, 5.3	$5x10^{-5}$
21	400	S. typhimurium 1	Tc^r	100,3.6	Tc^r	100, 3.6	$2x10^{-3}$
22	407	S. typhimurium nt	Tc^r,Gm^r,Cm^r, SSS^r,Dc^r	131.8,91.2,3,2.5	Tc^r,Gm^r,(Dc^r)	131.8, 91.2	$3x10^{-4}$
23	408	S. typhimurium nt	Tc^r,Gm^r,Cm^r, SSS^r,Dc^r	100,95.5,2.9,2.6	Tc^r,Gm^r,(Dc^r)	100, 95.5	$7x10^{-2}$
24	409	S. typhimurium nt	Tc^r,Gm^r,Cm^r, SSS^r,Dc^r	100,95.5,2.9,2.6	ND	ND	ND
25	414	S. typhimurium 1	Tc^r	131.8,91.2,4.6,4.4	Tc^r	131.8, 91.2	$1x10^{-3}$
26	415	S. typhimurium 1	Tc^r,Gm^r,SSS^r,Dc^r	131.8,91.2,2.7,2.5	Tc^r,Gm^r,(Dc^r)	131.8, 91.2	$4x10^{-2}$
27	416	S. typhimurium 1	Tc^r,Gm^r,SSS^r,Dc^r	131.8,91.2,3,2.5	Tc^r,Gm^r,(Dc^r)	131.8, 91.2, 3	$6x10^{-3}$

Tc – tetracycline Km – kanamycin Sm – streptomycin Am – ampicillin Amo amoxicillin Cm – chloramphenicol Dc – doxicyclin SSS – sulfamide–

FENOTYPES	NO. STR AINS	%
Tc^r	8	53.3
Tc^r, Km^r	1	6.6
Tc^r, SSS^r	3	20
Tc^r, Am^r, Cm^r	1	6.6
Tc^r, Km^r, Sm^r	1	6.6
Tc^r, Am^r, Cm^r, Amo^r, Dc^r (My)	1	6.6

Table 1.1 *Salmonella enteritidis: Resistance fenotypes*

FENOTYPES	NO. STRAINS M	M+O
Tc^r	4	4
Tc^r, Km^r	1	0
Tc^r, SSS^r	0	3
Tc^r, Am^r, Cm^r	0	1
Tc^r, Km^r, Sm^r	0	1
Tc^r, Am^r, Cm^r, Amo^r, Dc^r (My)	0	1

Table 1.2. *Salmonella enteritidis: "tet" resistance determinants*

FENOTYPES	NO. STRAINS	%
Tc^r	3	25
Tc^r, Km^r	1	8.3
Tc^r, SSS^r	1	8.3
Tc^r, SSS^r, Sm^r	2	16.6
Tc^r, SSS^r, Gm^r, Dc^r	2	16.6
Tc^r, SSS^r, Gm^r, Dc^r, Cm^r	3	25

Table 2.1. *Salmonella typhimurium: Resistance fenotypes*

FENOTYPES	NO. STRAINS M	O	M+O
Tc^r	0	1	1
Tc^r, Km^r	1	0	0
Tc^r, SSS^r	0	0	0
Tc^r, SSS^r, Sm^r	2	0	0
Tc^r, SSS^r, Gm^r, Dc^r	0	0	2
Tc^r, SSS^r, Gm^r, Dc^r, Cm^r	0	0	3

Table 2.2. *Salmonella typhimurium: "tet" resistance determinants*

Conclusions

1. Within avian *Salmonella* strains under analysis the high frequency self - transferable character of the resistance to tetracycline was found.

2. The molecular analysis evidenced the prevalence of the determinants -O and M types - of the resistance to tetracycline that induced resistance mechanisms by ribosomal protection.

3. Several genetic Tc^r determinants may co-exist in *Salmonella* strains, as demonstrated by the concomitant presence of determinants O and M in 16 / 27 - 59.2 % of the strains analysed.

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МОЛЕКУЛАРНА КАРАКТЕРИЗАЦИЈА НА РЕЗИСТЕНЦИЈАТА НА ТЕТРАЦИКЛИН НА НЕКОИ АВИЈАРНИ СОЕВИ НА SALMONELLA TYPHIMURIUM И SALMONELLA ENTERITIDIS

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Генетскиот скрининг преку хибридизација со тет М и тет О ДНК проби го покажаа, кај соевите на салмонела изолирани во 1996 година, следното: 14/15 (93.3%) од Salmonella enteritidis соевите и 9/12 (75%) од S. typhymurium соевите ја поседуваат тет М детерминантата и 10/15 (66.6%) од Salmonella enteritidis соевите и 7/12 (58.3%) од S. typhymurium соевите исто така ја поседуваат тет О детерминантата.

Скринингот со ПЦР техниката за која користевме прајмери добиени од Елизабет Шаслус Данела, ИНРА Турс, Франција и со која се докажуваат заедничките фрагменти за А, Б, Ц, Д, Е детерминантите, беше негативен.

Присуството на тет М и тет О детерминантите кои ги кодираат механизмите за резистенција преку рибозомска заштита против сите типови на тетрациклини (од првата генерација и аналогните од втората генерација) можат да бидат поврзани со генетско пренесување од Грам-позитивните бактерии.

Во тестот на коњугација со E. coli БМ21 (gyr A, Nalr) резистенцијата на тетрациклин покажа висока трансфер фреквенција што сугерира за можноста од висок епидемиолошки ризик. Потеклото на соевите - три Романски географски региони - сугерираат дека М и О детерминантите за резистенцијата на тетрациклин се широко распространети меѓу S. typhimurium и S. enteriditis.